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Electrochemiluminescence and Immunochematographic Analysis of Samples Collected by the Canadian Integrated Biochemical Agent Detection System at Joint Field Trials IV

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**ELECTROCHEMILUMINESCENCE AND
IMMUNOCHROMATOGRAPHIC ANALYSIS OF SAMPLES
COLLECTED BY THE CANADIAN INTEGRATED
BIOCHEMICAL AGENT DETECTION SYSTEM
AT JOINT FIELD TRIALS IV**

by

R.E. Fulton, L. Stadnyk, M. Spence, J. Ho, and C. Boulet

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ABSTRACT

This report documents the analysis of samples taken at Joint Field Trials (JFT) IV for two biological agent simulants, *Bacillus subtilis* var. *niger* ssp. *globigii* (BG) and *Erwinia herbicola* (EH), by two different identification technologies, electrochemiluminescence immunoassay (ECLA) and immunochromatographic assay (ICA). In each of 25 trials, BG or EH was disseminated and then collected by one or more CIBADS collector systems, which consisted of two prototype CIBADS Sentry units located at different positions on the test grid and a Concept Demonstrator unit located outside the grid. On analysis of samples by ECLA, BG and EH were identified in 100% of trials in which BG and EH, respectively, were disseminated. The amount of simulant in samples was estimated from standard concentration curves. BG was found to be present in samples in concentrations ranging from approximately 7×10^4 to 7×10^6 cfu/ml; the concentration of EH in samples ranged from approximately 1×10^3 to 1×10^4 cfu/ml. For a given collection period, the amount of simulant identified by ECLA in samples was positively correlated with the amount of simulant collected by referee samplers. On analysis of samples by ILA, BG tickets produced "false positive" reaction lines when tested with CIBADS collection fluid (water) and, although shown to be sensitive enough to detect BG in the concentrations estimated to be present in samples, these tickets were declared unreliable. EH tickets did not react non-specifically when tested with water but were shown to be insufficiently sensitive to detect EH in the concentrations estimated to be present in samples. The implications of the findings documented in this report are discussed.

EXECUTIVE SUMMARY

Title

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Electrochemiluminescence and Immunochematographic Analysis of Samples
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Introduction

The Canadian Forces (CF) have a requirement to be able to conduct military operations under field conditions where there is a threat of use of biological weapons. To operate under such conditions, effective systems for detection of biological agents are required to provide warning in time for soldiers to don protective equipment. In addition, systems for identification of the biological agent are required in order that appropriate medical countermeasures can be initiated. To meet these requirements, a major research and development effort was initiated in autumn 1995 at Defence Research Establishment Suffield (DRES) for development of the Canadian Integrated BioChemical Detection System (CIBADS). The goal of this project was to provide a field-portable integrated chemical and biological agent detection and identification system for the CF by the year 2000.

The development of CIBADS has progressed under a number of phases beginning with the construction of a Concept Demonstrator model which was successfully demonstrated at international field trials in 1995. In these trials, near real time detection of biological agents was achieved by use of a Fluorescence Aerodynamic Particle Sizer (FLAPS). The most recent version of CIBADS is a Sentry system which incorporates both biological and chemical detection capabilities in one autonomous unit.

The final phase of the CIBADS project includes incorporation into the CIBADS Sentry system of automated biological agent identification capabilities. After review and evaluation of a number of different identification technologies, a decision was taken to incorporate antibody-based immunochematographic assay (ICA) as the first generation identification technology for CIBADS. Accordingly, at

Joint Field Trials (JFT) IV, conducted at Dugway Proving Ground (DPG) in autumn 1997, several technologies were evaluated for efficacy in identification of biological agents collected by two prototypes of the CIBADS Sentry system, CIBADS V96 and CIBADS V97, and a CIBADS Concept Demonstrator unit. In this report, we describe the analysis of samples taken at JFT IV for the two biological agent simulants, *Bacillus subtilis* var. *niger* ssp. *globigii* (BG) and *Erwinia herbicola* (EH), by two different identification technologies, electrochemiluminescence immunoassay (ECLA) and ICA.

Results

Samples collected by three different CIBADS collectors located at different positions on and outside the JFT IV trial grid were tested by ECLA and ILA for the presence of aerosolized simulants, BG and EH. On analysis of samples by ECLA, BG and EH were identified in 100% of trials in which BG and EH, respectively, were disseminated. The amount of simulant in samples was estimated from standard concentration curves. BG was found to be present in samples in concentrations ranging from approximately 7×10^4 to 7×10^6 cfu/ml; the concentration of EH in samples ranged from 1×10^3 to 1×10^4 cfu/ml. For a given sample collection period, the amount of simulant in samples identified by ECLA was positively correlated with the amount of agent collected by DPG referee samplers, suggesting that the cloud presented to the referee samplers was a good representation of the cloud presented to the CIBADS samplers. On analysis of samples by ILA, it was found that BG tickets produced "false positive" reaction lines when tested with CIBADS collection fluid (water). For this reason, even though BG tickets were shown to be sensitive enough to detect BG in the concentrations estimated to be present in samples, these tickets were declared unreliable. EH tickets, on the other hand, did not react non-specifically when tested with water but were shown to be insufficiently sensitive to detect EH in the concentrations estimated to be present in samples.

Significance of Results

The results of this work demonstrate that ECLA can be successfully used as a laboratory-based technology to identify biological agent simulants such as BG and EH in air samples collected by CIBADS. Under the conditions operational at JFT IV, BG concentrations in samples were well within the limits of detection (LOD) of the ECLA for BG; EH concentrations, however, were close to the LOD for EH. Results also demonstrate that referee samplers, when placed at tower locations in close proximity to test samplers (as at JFT IV), provide an estimate of simulant cloud concentration which is representative of the cloud concentration presented to the test samplers at the corresponding tower. Results suggest that there is a higher probability of identifying agents in samples when redundant samplers are used, compared with reliance upon a single collector for provision of test samples. Although ICA technology has been selected as the first generation identification technology for CIBADS, results from the evaluation of ICA in this study were disappointing, as tickets used to assess JFT IV samples fell short of requirements for specificity i.e., BG tickets, and sensitivity i.e., EH tickets. It is unclear whether these problems were batch-specific and originated in the manufacturing process or whether problems are inherent in the ICA technology.

Future Goals

Further work is required in the development and field assessment of ICA technology as the first generation identification technology for CIBADS. In this regard, further batches of simulant ICA tickets from the same manufacturer that produced the JFT IV tickets, will be evaluated. In addition, prototype ICA tickets currently under development by a Canadian manufacturer will be assessed as they become available in the future.

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INTRODUCTION

The Canadian Forces (CF) are required to be able to conduct military operations under field conditions where there is a threat of use of biological weapons. To operate under such conditions, effective systems for detection of biological agents are required to provide warning in time for soldiers to don protective equipment. Furthermore, systems for identification of the offending agent must be in place so that the effects of exposure can be mitigated through implementation of appropriate medical countermeasures. The CF envisage deployment of an integrated system for detection and identification of chemical and biological agents which would consist of a network of remote point detectors (sentries) with the capability of autonomous operation, near real time detection, and rapid identification of all threat chemical and biological agents. To meet these requirements, a major research and development effort was initiated in 1995 at Defence Research Establishment Suffield (DRES) for the development of the Canadian Integrated BioChemical Agent Detection System (CIBADS). The goal of the CIBADS project is to develop and produce a field-portable integrated chemical and biological agent detection system for the CF by the year 2000. During the initial stages of the CIBADS project, a Concept Demonstrator model of CIBADS, consisting of a mobile laboratory containing modular units which incorporated the basic elements of environmental aerosol detection, collection, and agent identification, was built [1]. At Joint Field Trials (JFT) II at Dugway Proving Ground (DPG) in 1995, the CIBADS Concept Demonstrator was successfully trialed and the concept of integrated biological detection and identification was proven [2]. At these trials, near real time detection of biological agent simulants was achieved by use of a Fluorescent Aerodynamic Particle Sizer (FLAPS) (TSI Inc., Minneapolis, MN). In 1996, a stand-alone unit which could operate autonomously in the field, the CIBADS II Version 96 (V96) CB Sentry, was built and trialed successfully at JFT III at DPG. This system incorporated FLAPS 2, an improved generation of FLAPS, an XMX aerosol sampler and particle concentrator, and a radio modem for remote control of operations and data transmission. Performance of CIBADS V96 at JFT III demonstrated that aerosolized biological simulants could be automatically detected in the field at concentration levels consistent with the sensitivity

requirements of currently available identification technologies. The most recent version of the CIBADS CB Sentry, CIBADS II V97, underwent field testing at JFT IV at DPG in October and November, 1997. This system was modified from CIBADS V96 and incorporated both biological and chemical detection capabilities in one autonomous unit. In addition, it was fitted with an XMX particle concentrator, a Large Volume Air Sampler (LVAS), a Global Positioning System (GPS), meteorological equipment, and radio communication capabilities.

The final phase of the CIBADS project includes incorporation of automated biological agent identification capabilities. As a first step in selection of identification technologies for incorporation in CIBADS, surveys of off-the-shelf and developmental identification technologies were conducted [3] and several technologies were evaluated under field conditions. Hence, the Light Addressable Potentiometric System (LAPS) or Threshold[®] device (Molecular Devices Corp., Menlo Park, CA) and the Origen[®] Electrochemiluminescence (ECL) analyser (IGEN Inc., Gaithersburg, MD) were optimized for identification of biological agent simulants and tested at JFT II [2]. In 1997, a decision was taken to use immunochromatographic assay (ICA) as the first generation identification technology to be incorporated into CIBADS. Shortly thereafter, work on development of an automated ICA ticket reader for integration with CIBADS was initiated.

Canadian detection and collection equipment tested at JFT IV included CIBADS II Version 96 (V96), CIBADS II Version 97 (V97), and the CIBADS Concept Demonstrator unit (CDU) as previously deployed at JFT II [2]. Identification technologies evaluated at JFT IV included LAPS immunoassay [4], electrochemiluminescence immunoassay (ECLA), and ICA. In this paper, we describe the analysis of samples taken at JFT IV for two bacterial simulants, *Bacillus subtilis* var. *niger* ssp. *globigii* (BG) and *Erwinia herbicola* (EH) by ECLA and ICA. ECLA proved to be a sensitive and reliable technique for identification of BG and EH in collected air samples. ICA, on the other hand, was shown to be unreliable for the analysis of BG in samples and insufficiently sensitive for the identification of EH in samples. Results of this study indicate that further work is required to determine whether problems identified with the use of ICA tickets originate with poor quality

control at the manufacturing level or whether problems are inherent in the ICA technology.

METHODS AND MATERIALS

Trial Configuration at Dugway Proving Ground

Outdoor field trials were conducted at the Tower Grid, West Desert Test Centre, Dugway, Utah, 8 October - 7 November, 1997. Trials took place during periods of darkness and only when acceptable meteorological conditions prevailed. Procedures followed were those provided in the Abbreviated Test Plan for JFT IV [5] which, briefly, were as follows. Biological aerosols were generated at a desired aerosol concentration of 5-15 Aerosol Cloud Particles Per Liter of Air (ACPLA) from a point source located 600 to 1200 m upwind. Reference towers, consisting of referee samplers, were placed approximately 10-15 m apart along a "sampling line" approximately 70 m in length. Test equipment was placed on either side of each reference tower at a radial distance of 1-3 m from the reference tower centre. In order that the cloud characteristics presented to the referee equipment represented as closely as possible those presented to the test equipment, the air inlet of test items was positioned at the same height as that of the aerosol characterizing reference samplers. To be considered valid, data from test equipment could be compared only with data from referee samplers on the same reference tower [5]. Reference instrumentation consisted of New Brunswick slit-to-agar samplers (NBSS) and Aerodynamic Particle Sizers (APS). Data collected by this instrumentation included particle size distribution, time of aerosol cloud arrival, cloud particle concentration and duration, and uniformity of aerosol content. Raw data collected by the referee samplers was provided by DPG to trial participants following the trials. Analysis of referee data in relation to data generated by the CIBADS detector system is expected to be the subject of a separate publication.

JFT IV Detection and Collection Systems

DRES deployed two CIBADS Sentry systems for test and evaluation at JFT IV, CIBADS V96 (equipped with XMX sampler) (V96:XMX), CIBADS V97

(equipped with LVAS) (V97:LVAS), and the CIBADS CDU. Each Sentry functioned autonomously and was capable of automated, continuous operation. V96:XXM and V97:LVAS were positioned at reference towers 2 and 3, respectively. The CDU housed detection and collection equipment and served as an operations command and control centre for the CIBADS team. The CDU was situated outside of, but in close proximity to, the test grid.

CIBADS II V97

Detection and collection components of this system consisted primarily of a FLAPS biological detector, a chemical agent sensor, a XMX particle concentrator, and a LVAS. Additionally, the system included GPS equipment and instrumentation for meteorological monitoring. The FLAPS, which operates on a principle of aerodynamic particle sizing coupled with particle fluorescence measurements, interrogated the air for the presence of bioaerosols and, when a biological event was detected, the LVAS was triggered to initiate sample collection. The LVAS collected 1000 L of air per min and concentrated the contents into 1 ml distilled water. The LVAS was remotely controlled from the CDU and samples were typically collected for a period of 10 min following the initial collector trigger. When a biological event occurred, alarm and event details, including raw data, were transmitted to the CDU within 15 sec of the biological event. At the end of the sampling period, a message was received in the CDU that the sample was ready for retrieval. One person was then dispatched to remove the collection tube containing the sample, to replace it with an empty collection tube in preparation for the next sampling event, and to transport the collected sample to the CDU for identification.

CIBADS II V96

The CIBADS II V96 system was positioned on the grid "sampling line" at an estimated distance of 15-16 m from the CIBADS II V97 system [5]. CIBADS II V96 was, essentially, a modified version of the CB Sentry deployed at JFT III, the prototype model upon which the CIBADS II V97 was built. The detection components of CIBADS II V96 consisted primarily of a FLAPS biological detector,

a XMX particle concentrator, and a XMX liquid sampler, the latter being a prototype microfluidics system developed by Dycor Industrial Research Inc. (Edmonton, AB). The CIBADS II V96 system also included GPS equipment, meteorological monitoring instrumentation, and was linked by radio modem to the CDU for transmission of information and control of Sentry operation. The FLAPS monitored the air for the presence of bioaerosols and, in the event that a biological event occurred, the XMX collector was remotely triggered to collect a sample. The XMX sampler collected 1 L of air per minute into a fixed volume of 20 ml distilled water; sampling times varied from trial to trial. At the end of the sampling period, a person was dispatched to retrieve the collected sample, replace the collection tube, and transport the sample to the CDU for identification.

Concept Demonstrator Unit

The CDU was essentially the CIBADS Concept Demonstrator trailer unit as developed for the JFT II trials at DPG in 1995. The CDU was positioned outside of the Tower Grid and, in addition to serving as the operation centre for the CIBADS team, housed the computers for communication with, and control of, the CB Sentries. The CDU also served as a laboratory, where some assays for identification of simulant agents in collected samples were performed. In addition, the CDU was equipped with a FLAPS biological detector and a LVAS which was pre-programmed to collect 3 min (3 ml) samples in series. When a biological event was detected by the FLAPS, the LVAS was manually engaged by CDU personnel. Typically, the LVAS was switched on when a "trial mode" was called by DPG and samples were collected throughout the biological event. A sample collected close to the opening of the trial mode was arbitrarily designated by CDU personnel as a "background control" sample.

Biological Agents Disseminated

Trial simulants, BG and EH, were disseminated as point source wet aerosols by Micronair generators (Micronair Ltd., Sandown, Isle of Wight, UK). The procedure for preparation of BG and EH for use in field trials was provided

previously by DPG [D. Martin, DPG, personal communication] and, briefly, is as follows. Dry BG, obtained in 1992 from Pine Bluff, AK, was mixed with deionized water in a ratio of 50 g BG: 1 liter water. The concentration of BG for aerosolization in field trials was approximately 1×10^{10} colony forming units (cfu) per liter. EH was purchased from American Type Culture Collection (Manassas, VA) and subcultured at DPG in tryptic soy broth. A concentration of approximately 1×10^9 cfu per ml was used for field trials.

Test Samples

Samples generated by CIBADS V97:LVAS were assayed by ICA at DPG immediately following each trial. All samples were stored at -70°C while at DPG and, following the trials, were transported to DRES for further analyses. At DRES, samples collected by CIBADS V96:XXM, V97:LVAS, and CDU:LVAS samplers were assayed in parallel by ECLA. In addition, those samples which had not been tested at DPG by ICA i.e., V96:XXM and CDU:LVAS samples, were assayed by ICA at DRES.

Electrochemiluminescence Immunoassay

Antibodies

A detailed description of the antibodies developed for analysis of agent simulants by ECLA has been previously published [2]. In brief, BG antibodies were developed at DRES by hyperimmunization of rabbits with inactivated, washed BG cells. EH antibodies (goat and rabbit) were provided by the Naval Medical Research Center (NMRC) (formerly Naval Medical Research Institute (NMRI), Bethesda, MD). BG antibodies were purified by Fast Performance Liquid Chromatography (Pharmacia Inc, Dorval, QU) on protein G columns by SciLab Consulting Inc (Medicine Hat, AB); EH antibodies were purified by NMRC. Rabbit anti-BG IgG and goat anti-EH IgG were conjugated at DRES with Origen[®] biotin-LC-sulfo-NHS ester (sulfosuccinimidyl-6-(biotinamido) hexanoate) (IGEN Inc.). Similarly, rabbit anti-BG and rabbit anti-EH were conjugated at DRES with Origen[®] TAG[®]-NHS ester ([bis (2,2"-bipyridine) (4-methyl-4' (N-succinimidylloxycarbonyl-propyl)-2,2'-

bipyridine) ruthenium (II) hexafluorophosphate]) (IGEN Inc.). The protein concentrations of biotin-labelled rabbit anti-BG and goat anti-EH IgG were estimated at 200 ug/ml and 230 ug/ml, respectively. Protein concentrations of TAG^R-labelled rabbit anti-BG IgG and rabbit anti-EH IgG were estimated at 251 ug/ml and 190 ug/ml, respectively.

Assays

ECLA for BG and EH were performed by ECL analyser (IGEN Inc.), as previously described [2]. Samples from V97:LVAS, V96:XXM, and the CDU: LVAS were analysed, in parallel by trial number, in triplicate, for BG and EH, respectively. Triplicate known positive and negative control samples were included for each carousel run of the ECL analyser. Positive controls for BG assays consisted of BG at known protein concentrations of 10, 1.0 and 0.1 ug/ml; EH assay positive controls consisted of EH at 2.6×10^4 , 2.6×10^3 , and 2.6×10^2 cfu/ml. Negative controls consisted of assays which contained all immunoassay reagents except antigen.

A typical immunoassay was conducted as follows. Biotin- and TAG^R-labelled antibodies were combined in a cocktail mixture to achieve a final protein concentration of 3 ug per ml biotin-labelled antibody and 1 ug per ml TAG^R-labelled antibody. In a separate tube, 50 ul of antibody cocktail was added to 50 ul of test sample and the mixture was incubated for 60 min at room temperature on vortex mode of the ECL analyser. Fifty ul of a stock mixture of streptavidin-coated magnetic polystyrene beads (1 mg per ml in distilled water) (M-280 streptavidin Dynabeads^R) (DynaL Inc., Lake Success, NY) was then added and incubation was resumed with vortexing for a further 30 min. At the end of this step, 200 ul of Origen^R assay buffer (IGEN Inc.) was added and the ECL count was quantitated immediately by ECL analyser. Diluent buffer for ECL assays was phosphate buffered saline (PBS), pH 7.4 (Sigma Chemical Co., St Louis, MO) containing 1.0 % bovine serum albumin (Sigma) and 1.0 % Tween-20 (Sigma).

Data Acquisition and Analysis

Data was generated by the ECL analyser in the form of ECL counts and imported into Microsoft Excel V5.0 for reduction and statistical analysis. The reduced data was further imported into Grapher™ (Golden Software Inc, Golden, CO) for plotting of graphs.

Immunochromatographic Assay

ICA for BG and EH were performed by ICA tickets purchased from Majesco Biologicals Inc. (Edison, NJ). Antibodies and antigens used in the manufacture of tickets were either gifts from NMRC or were provided to the manufacturer by DRES. The lower limits of detection (LOD) for the ICA tickets, as provided in the manufacture's specifications, was 1.0×10^5 cfu/ml for BG tickets and 2.3×10^6 cfu/ml for EH tickets. Quality control studies, including evaluations of sensitivity, specificity, and reproducibility were conducted at DRES prior to JFT IV (data not shown). In these studies, the LOD were found to be approximately 3.5×10^4 cfu/ml for BG tickets and approximately 6.9×10^6 cfu/ml for EH tickets. Furthermore, tickets were specific for the homologous simulant i.e., there was no cross-reactivity with heterologous simulants ovalbumin and MS-2, and assays were reproducible from ticket to ticket.

Due to the limited availability of BG and EH tickets, it was not possible to perform ICA on samples from all three collectors, either while at DPG or in parallel at DRES following the trials. Therefore, V97:LVAS samples were tested by ICA at the DPG trial site only, while V96:XXM and CDU:LVAS samples were tested only at DRES following the trials.

ICA was performed by placing 200 μ l of test sample into the receptacle well of the ICA ticket, one ticket for BG and one for EH, and allowing the chromatographic reaction to "develop" for 15 min at room temperature. Tickets were then qualitatively scored (positive or negative) for the presence of simulant. For ICA conducted at the trial site, tickets developed at DRES prior to the trials, with known concentrations of BG (3.5×10^6 , 3.5×10^5 and 3.5×10^4 cfu/ml) and EH (2.6×10^8 , 2.6×10^7 and 2.6×10^6 cfu/ml), were used as reference standards. For

assays performed at DRES following the trials, positive controls consisted of similar preparations of BG and EH and negative controls consisted of ICA buffer, PBS (120 mM NaCl, 2.7 mM KCl, 10 mM phosphate buffer, pH 7.4) (Sigma) containing 0.1% Triton X-100.

Immunoassay Control Antigens

Bacillus subtilis

Dry BG was obtained from DPG and consisted of a combined blend of several production lots of BG with a viable cell concentration of approximately 1×10^{11} cfu/g (B. Harper, DPG, personal communication). Stock solutions of BG were prepared by resuspending 0.1 g dry BG in 10 ml distilled water.

Erwinia herbicola

A stock culture of EH, strain 33243 (ATCC) was obtained from DPG and grown at DRES. A detailed description of methods used in preparation of EH antigen has been previously published [2].

Protein Assays

Protein concentrations were determined by bicinchoninic acid (BCA) protein assay in accordance with instructions provided by the supplier (Pierce Inc., Rockford, IL). In addition, protein concentrations were estimated from the molar absorptivity coefficients of rabbit IgG and goat IgG by the formula $E^{1\% / 1\text{cm}} \times C = A_{280}$ [6] [7].

RESULTS

Conduct of Trials

There were 25 trials in total, conducted over a period of 16 non-consecutive days from 22 October to 6 November 1997. The trial schedule and trial windows² are

¹ $E^{1\% / 1\text{cm}}$ = absorption of a 1% solution through a path length of 1 cm; C = concentration; A_{280} = absorption at 280 nm. The $E^{1\% / 1\text{cm}}$ of rabbit IgG at 280 nm was approximated at 13.6; the $E^{1\% / 1\text{cm}}$ of goat IgG at 280 nm was estimated from the molar absorptivity coefficient of horse IgG = 13.8.

² Trial window: time period during which test grid was in "active test mode", as announced by DPG

shown in Table I. Trials occurred in nine sessions (I - IX) in which agent disseminations were not separated in time by more than several hours. Trials 1 and 2 were intended for pre-trial calibration of equipment and procedures. For this reason, these trials were "unscored" and the identity of the disseminating agent was announced prior to spraying. For all subsequent trials, test results were "scored" and the identity of disseminated agent was unknown at the time of the trial. Information for each trial on identity of material sprayed and results from on-grid referee instrumentation were made available by DPG following completion of JFT IV.

Electrochemiluminescence Immunoassays of Trial Samples

Test Results

Samples collected by CIBADS II V96, CIBADS II V97, and the CDU were analyzed by ECLA for the presence of BG (Tables II) and EH (Table III). For each test sample, the mean ECL signal (S^3) was determined and the ratio of S to the mean background noise (N^4) (S/N) was calculated. The value of S/N was used to determine whether a given sample was positive or negative for antigen. Test samples were considered positive if S/N was greater than 1.0; samples in which S/N was less than 1.0 were negative. The significance of each positive S/N was assessed by comparing the standard deviation on the ECL reading of the test sample with that obtained on the negative control sample. For S/N to be statistically significant, the value of S minus two standard deviations had to exceed the value of N . All values of S/N which did not meet this criterium were scored negative.

In the BG assay, all samples collected by the V96:XXM collector and the V97:LVAS were positive for BG. Similarly, BG was identified in all but one sample (trial 10, sample no. 64) collected by the CDU: LVAS. In the EH assay, EH was identified in one or more samples collected by one or more of the collectors in trials 2, 5, 10, 12, 13, and 17-21.

³ S = mean ECL of triplicate tubes of test sample

⁴ N = mean ECL of triplicate tubes of negative control sample + 2 standard deviations

Concentrations of Simulants in Samples

The concentration of BG and EH in test samples was estimated from data relating ECL to known concentrations of BG and EH, respectively, in positive control samples which were included in each carousel run of the ECL analyser. Best fit standard curves and regression formulae for positive control data were determined by a curve fitting program (Grapher™). For both BG and EH, the relationship of ECL to concentration was best described by the log regression formula $[y = A + B \cdot \log x]^2$. The concentration of simulant in test samples was estimated by substituting the values for x and y from the positive control data series into the regression formula and solving for A and B, followed by substitution of the values for A, B, and observed y (ECL of test sample) and solving for x. Concentrations of BG in test samples in which BG was identified were expressed in both ug/ml (calculated from the log regression formula) and cfu/ml³ (Table IV). Concentrations of EH in test samples shown to contain EH were calculated from the log regression formula and expressed in cfu/ml only (Table V). Concentrations of BG in individual test samples were also estimated directly from histograms relating ECL count to standard regression curves for positive control samples of BG (Figures 1- 6). Estimates of BG concentration in V96:XX samples ranged from a low of 0.078 ug/ml, corresponding to approximately 7.8×10^4 cfu/ml in trial 2, to a high of 1.05 ug/ml or 1.1×10^6 cfu/ml in trial 8. Concentrations in V97:LVAS samples ranged from 0.084 ug/ml or approximately 8.4×10^4 cfu/ml in trial 2, to 2.27 ug/ml or 2.3×10^6 cfu/ml in trial 16. Concentrations in CDU:LVAS samples ranged from 0.072 ug/ml or 7.2×10^4 cfu/ml in trial 18, to 6.77 ug/ml or 6.8×10^6 cfu/ml in trial 8 (sample LVAS 58) and 9.198 ug/ml or 9.2×10^6 cfu/ml in trial 2 (sample 15) (unscored sample).

EH concentration estimates ranged from trace amounts (4.5 to 7.2×10^2 cfu/ml) in isolated samples from trials 10, 12, 13, 18, 19, and 20 to concentrations

² $Y = A + B \cdot \log x$, where y = mean ECL count, x = concentration (ug/ml), A = y intercept (constant), and B = regression coefficient (slope)

³ 1 pg BG = approximately 1 cfu [8]

ranging from a low of 1.3×10^3 cfu/ml to a high of 0.8×10^4 cfu/ml in samples from trials 2, 5, 17, and 21.

Immunochromatographic Assays of Trial Samples

All samples that were tested by ECLA were also tested by ICA. Results of ICA for BG and EH for test samples collected by the V96:XXM, V97:LVAS and the CDU: LVAS are presented in Table VI. With the exception of the V96:XXM sample from trial 19, all samples tested by ICA were positive for BG. Conversely, all samples tested by ICA were negative for EH.

From the estimated concentrations of BG in test samples (Table IV), it was observed that BG was present in most samples at concentration levels well in excess of the LOD of BG tickets. However, examination of the data in Table V indicates that EH detected in test samples by ECLA was consistently present in concentrations well below the LOD of the EH tickets. These observations indicate that, while the LOD of the BG tickets was sufficient for detection of BG in the JFT IV test samples, the sensitivity of the EH tickets was inadequate for detection of EH in these samples.

Electrochemiluminescence and Immunochromatographic Analyses of Water Samples

During screening of samples by ICA at DRES following JFT IV, a number of samples consisting of the water used as collection fluid for the CIBADS samplers were included as negative test controls. It was found that these water samples produced positive test lines on most BG tickets: six of seven replicate assays of CIBADS NANOpure[®] water (NANOpure II system, Sybron, Barnstead/Thermolyne Corp., Dubuque, IO) were positive on BG tickets. When the same water samples were tested on EH tickets, four of four replicate assays were negative.

To investigate this phenomenon further, water samples from the CIBADS and from various sources and locations at DRES were tested by ECLA for BG and EH. In addition, water samples were tested by ICA on BG tickets, both before and after

buffering with sodium phosphate buffer (Oxoid Inc., Nepean, ON)⁷. The results of this study are tabulated in Table VII. By ECLA, all water samples were negative for EH. Similarly, water samples were negative by ECLA for BG, with the exception of DRES Building 67 NANOpure^R water, DRES Building 1 tap water, and the CIBADS NANOpure^R water (both before and after a filter change), which were positive for trace amounts of BG. To elucidate whether positive test results obtained with water could be attributed to pH, the pH of water samples was measured and samples were tested by ICA both before and after buffering with sodium phosphate buffer. It was found that the pH of all water samples was approximately neutral, with the exception of tap water which had a pH of 8.0. However, while all samples were positive by ICA for BG prior to buffering, these same samples were negative for BG after they were buffered with sodium and phosphate salts.

Due to the fact that BG tickets were subject to "false positive" test reactions associated with the use of unbuffered samples and the EH tickets were insufficiently sensitive to detect EH at the concentrations used in the trials, no further analyses of JFT IV ICA results were pursued.

Comparison of Simulant Concentrations in Referee and CIBADS Samples

The purpose of the referee data was to provide information on cloud concentration and on cloud arrival and departure times at each of the tower sites during trials. To assess how closely the simulant content of the cloud presented to the referee samplers reflected the simulant content of the cloud presented to CIBADS V96:XXM and V97:LVAS collectors, the amount of simulant in samples collected by referee samplers was compared to the amount of simulant collected by the CIBADS collectors, as follows. At reference tower 2, where CIBADS V96:XXM was located, the concentration of simulant in NBSS samples, as calculated from DPG reference data, was evaluated in relation to the concentration of simulant in V96:XXM samples, as estimated from ECLA data. For each trial in which samples were collected by CIBADS V96:XXM, total ACPLA was calculated for the period

⁷ Dulbecco "A" PBS: 8 g/liter sodium chloride, 0.2 g/liter potassium chloride, 1.15 g/liter disodium hydrogen phosphate, 0.2 g/liter potassium dihydrogen phosphate

of time that the XMX sampler was switched on (Table VIII). Total ACPLA in these samples was then plotted as a function of the simulant concentration in cfu/ml collected by V96:XXM. The resulting regression formula and the coefficient of correlation were then determined. A positive linear relationship ($R = 0.72$) was observed between total ACPLA in NBSS referee samples and cfu/ml in V96:XXM samples collected at tower 2. In a similar manner, at reference tower 3, where CIBADS V97:LVAS was located, total ACPLA was calculated for the period of time that the LVAS was operational (Table IX) and total ACPLA was plotted as a function of the simulant concentration in cfu/ml collected by the V97:LVAS. A similar positive correlation between ACPLA in NBSS referee samples and cfu/ml in V97:LVAS samples was observed ($R=0.85$).

Simulants Identified by Electrochemiluminescence in Comparison to Identity of Agents Sprayed

During JFT IV, EH was disseminated in trials 2, 5, 17 and 21. In all other trials (trials 1, 3-4, 6-16, 18-20, and 22-25), BG was the simulant sprayed. In none of the 25 trials comprising JFT IV were both BG and EH disseminated simultaneously from the same slurry. Table X summarizes the agents sprayed and the agents identified by ECLA in samples collected by V96:XXM, V97:LVAS, and CDU:LVAS. The simulant sprayed was identified by ECLA in one or more of the samples collected by the V96:XXM, the V97:LVAS, and/or the CDU:LVAS in 25/25 trials, representing an overall correct identification rate in trials of 100%. Conversely, in none of the trials was neither BG nor EH identified i.e., 0% false negative identification rate. The simulant sprayed was identified in 17/17 samples (100%) collected by the V96:XXM and in 21/21 samples (100%) collected by the V97:LVAS. Similarly, in samples taken by the CDU:LVAS, the agent sprayed was identified in one or more samples in 21/21 trials (100%). However, in 10/25 trials (40%), not only was the simulant that was sprayed identified, but also trace amounts of the other JFT IV simulant were identified. Thus, in 3/17 V96:XXM samples (17.6%) and 6/21 V97:LVAS samples (28.6%), both BG and EH were identified;

similarly, both BG and EH were identified in one or more samples collected by the CDU:LVAS in 6/21 trials (28.6%).

DISCUSSION

In all trials in which BG was disseminated, BG was correctly identified by ECLA in samples collected by one or more of the CIBADS collectors. Similarly, in all trials in which EH was the disseminated simulant, EH was identified in one or more trial samples. Accordingly, the simulant disseminated was identified in 100% of the trials comprising JFT IV. However, in several trials not only was the simulant that was sprayed identified but also low levels of the other trial simulant were also identified. Hence, low levels of BG were detected in samples collected by V96:XXM, V97:LVAS, and/or CDU:LVAS collectors in all four of the EH trials (trials 2, 5, 17 and 21). Similarly, low levels of EH were identified in samples from BG trials 10, 12 and 13 and 18-20.

There are a number of possibilities which could account for identification of BG in trials in which EH was the disseminated agent. Firstly, there may have been carry-over or secondary aerosolization of BG from the immediately preceding trials. This scenario is particularly credible since all four EH trials in which BG was identified were preceded closely in time by one or more BG trials conducted within the same trial session. Furthermore, the time interval between closing of the preceding BG window and opening of the window in which EH was sprayed was typically less than one hour (mean time 35 minutes). It is thus possible that low levels of BG were still airborne on the grid from the previous trial when the next trial window was opened. Secondly, there may have been BG in these samples originating from re-aerosolization of BG accumulated on the grid from past trials. It is well known that the grid at DPG where JFT IV was conducted is "heavily contaminated" with BG from numerous trials conducted at this site over previous years (anecdotal information). Unfortunately, there was no mechanism in place at JFT IV for differential identification of BG and EH in referee samples collected at the tower grids. Rather, an assumption was made that the identity of the biological material present in the referee samples was the same as that of the material

disseminated (J. Ho, DRES, personal communication). There is, therefore, no way of knowing whether low levels of BG were indeed present on the grid throughout most of the trials. Other possible scenarios which could account for identification of BG in EH trials include trial-to-trial contamination of aerosol generators and/or sample collector(s) and/or assay-to-assay carry-over of biological materials in the ECL analyser. As aerosolization of trial simulants at JFT IV was controlled by DPG personnel, the feasibility of trial-to-trial carry-over in aerosol generators cannot be readily assessed. However, contamination of the fluidics of the LVAS with BG during field trials has been previously observed and, as a result of this observation, DRES instituted a procedure for routine decontamination of the LVAS following each collection operation (M. Spence, DRES, personal communication). This decontamination procedure, which consisted of flushing the LVAS with 10% bleach for 2 min followed by a 10 min rinse with NANOpure[®] water, was in place during JFT IV. However, because the effectiveness of this procedure was not confirmed by ECLA, the occurrence of carry-over of immunoreactive BG antigen in the collector fluidics cannot be excluded. Another possibility is that there may have been tube-to-tube carry-over of BG antigen in the ECL analyser. This occurrence, however, is unlikely because the ECLA measurement cycle includes, between samples, an automated wash step with caustic cleaning fluid containing potassium hydroxide and triton-X detergent. Furthermore, the occurrence of tube carry-over of antigens was controlled for, but not observed, during system optimization experiments conducted on the ECLA analyser prior to JFT IV (data not shown).

Trace amounts of EH were also identified in some trials in which BG was the disseminated agent e.g., trials 10, 12, 13, and 18-20. The estimated concentration of EH in some of these samples, however, particularly those from trials 10, 12, and 13, was very close to the LOD of the ECLA for EH. This suggests that these readings may represent "false positive" test results which might have been eliminated had a larger number of assay replicates been included. Precision in the EH ECLA would, undoubtedly, have been improved had more sample replicates been used, however, this was not possible due to limitations in sample availability. Other factors which may have contributed to the identification of EH in BG trials include antigen carry-

over or re-aerosolization from immediately preceding trials, as discussed above. For example, trials 18, 19 and 20, in which trace amounts of EH were identified in some samples, followed closely in time trial 17 in which EH was the disseminated agent. There was 20 minutes between the closing of trial 17 and the opening of trial 18 and less than 30 minutes separating the opening and closing of successive trial windows from trials 18-20. However, as noted above, there were no mechanisms in place at JFT IV to assess the specific microbial content of reference tower samples and it is, therefore, impossible to know whether trace amounts of EH were indeed present on the grid during the trials in question.

For the reasons discussed above, it is unclear whether the identification by ECLA in some samples of extraneous simulant i.e., simulant other than that sprayed, reflects the true content of these samples, or, whether these reactions represent "false positive" test results. It is interesting to note that the BioDetector Device (BD) (Environmental Technologies Group, Baltimore, MD), which was used for assay of the same samples for BG, gave positive alarms for each trial in which BG was disseminated but did not give "false positive" alarms for trials in which EH was intentionally sprayed [4]. This observation gives credence to the interpretation that observations by ECLA of trace amounts of extraneous simulant in some samples represents true "false positive" reactions. Indeed, some low level ECLA determinations could have been eliminated by raising the cut-off threshold of the ECLA. This action, however, would have resulted in a reduction in the number of positive identifications of the disseminated simulant. Ideal background samples for determination of cut-off thresholds for ICLA in JFT IV would have been pre-trial air samples known not to contain simulant antigens pertinent to the JFT IV trials. Because such samples were not available, PBS was used to determine the assay cut-off threshold. While PBS serves as an adequate internal control for ECLA, its use as background control for air samples containing simulant does not take into account ECL signal derived from materials in the air other than simulant. This situation results in establishment of an ECLA threshold which is artificially low and a resultant assay sensitivity which is artificially high. At JFT IV, samples which were taken by the CDU:LVAS in timed series prior to a FLAPS alarm were intended for use as

background control samples (M. Spence, DRES, personal communication). However, the results of this study have shown that these samples were unsuitable for this purpose because of their high content of disseminated agent. Difficulties in selection of appropriate background samples for use in field trials have been previously encountered and discussed [2]. Further research is required to determine the best type of sample for use as background control for air samples, for use in establishment of assay sensitivity thresholds of CIBADS identification technologies.

BG and EH were identified in all trials in which BG and EH, respectively, were disseminated; however, in any given trial, BG or EH were not necessarily identified in all of the samples collected at each of the locations. This observation probably reflects true variance in concentration of simulant cloud at various locations on the grid at any given time during any given trial. This interpretation is supported by the observation that ACPLA in referee samples varied from reference tower to reference tower during any given collection period (data not shown). To identify an agent disseminated in any given trial at JFT IV, it was not necessary for all samples in that trial to be positive for a given agent; rather, identification of the agent sprayed could be made on the basis of one or more positive samples collected at one or more grid locations. This observation points out the benefit in redundancy in collectors for provision of test samples i.e., the advantage in use of two or more collectors situated at different locations rather than dependence on a single collector.

The concentration of BG in samples collected during BG trials was relatively high, ranging from 7×10^4 to 7×10^6 cfu/ml, in comparison to the concentration of EH in samples collected during EH trials, which ranged from 1×10^3 to 1×10^4 cfu/ml. Similarly, cloud concentrations of simulant at referee towers 2 and 3 were generally higher during BG trials than during EH trials: At tower 2, total ACPLA collected by the NBSS during the corresponding V96:LVAS sampling periods ranged from 50.9 to 5652.3 during BG trials. At tower 3, total ACPLA collected during corresponding V97:LVAS sampling periods ranged from 771.2 to 7826.5 during BG trials and 67.8 to 272.4 during EH trials. Reasons for the presence of BG on the test grid in higher concentrations than EH are unknown. The JFT IV test plan had specified a desired cloud concentration of 5-15 ACPLA and a minimum dwell time

of 5-6 minutes [5] for both BG and EH. However, EH may have been aerosolized at less than the desired optimal cell concentration compared to BG. Alternatively, BG and EH may have been aerosolized from similar slurry concentrations but the dynamics of BG and EH fallout in the environment may have resulted in differential dwell times. In general, BG concentrations in samples were well within the LOD of the ECLA for BG (approximately 4×10^4 cfu/ml). EH concentrations, however, were close to, or at, the LOD of the ECLA for EH (approximately 1×10^3 cfu/ml). Similarly, BG was present in samples at concentrations well within the LOD of the ICA (approximately 1×10^5 cfu/ml). Conversely, EH was present in samples at concentrations well below the LOD of the ICA for EH, estimated at 2.0×10^6 cfu/ml.

There was good positive correlation between the concentration of simulants in referee samples and in samples collected by V96:XXM and V97:LVAS. Coefficients of correlation (R) were 0.72 and 0.85 at towers 2 and 3, respectively. These statistics suggest that the simulant content of the cloud presented to the referee samplers was a reasonably good representation of the cloud presented to the CIBADS samplers. This correlation is high considering that the methods used for assay of samples collected by referee samplers were different from those used to assay the CIBADS samples. Furthermore, assays of referee samples were based on viability whereas the CIBADS samples were assayed by immunologically-based methods which detect both viable and non-viable organisms.

Immunochromatographic assays obtained for technology evaluation during JFT IV proved not to be useful as identification back-ups for the IGEN ECL analyser during these trials. Although the BG tickets were shown, pre-trial, to be sensitive enough to detect BG in the concentrations calculated to have been present in the JFT IV test samples, the tickets were shown, post-trial, to give "false positive" test reactions with water. Since water was used as the collection fluid for all samples taken at JFT IV, BG results obtained by ICA were suspect and, therefore, considered invalid. EH tickets, on the other hand, were not subject to "false positive" test reactions with water samples. However, the LOD of these tickets was shown, pre-trial, to be at least two logs below that required to detect EH in the concentrations calculated to be present in the JFT IV test samples. Because the ICA tickets

incorporate a buffering system, they should, theoretically, be useable with water-based samples. The occurrence of "false positive" reactions with water was not previously encountered by NMRC, the developers of the prototype BG ticket (C. Morgan, NMRC, personal communication). However, it is known that prototype ICA systems developed at NMRC are standardized on the use of PBS containing 0.1% TritonX-100 as the sample buffer.

In an effort to elucidate reason(s) for the observed reactivity of BG tickets with control water, experiments were conducted post-trial at DRES to determine, firstly, whether BG was indeed present in control water and, secondly, whether positive reactions on BG tickets could be attributed to conditions of pH. To investigate these possibilities, water samples from various DRES locations were tested by ECLA for the presence of BG and EH and, by ICA, both before and after buffering with sodium phosphate. Although none of the water samples was positive by ECLA for EH, several water samples, including the water used as collection fluid for CIBADS at JFT IV, showed trace amounts of BG by ECLA. As discussed above, it is possible these observations represent "false positive" test reactions rather than true sample content. The pH of water used as CIBADS collection fluid and water collected from various locations at DRES was approximately neutral. Furthermore, "false positive" reactions on BG tickets could be eliminated when water samples were buffered with sodium-phosphate salts. These observations suggest that "false positive" reactions on BG tickets were not associated with the H ion concentration of the water i.e., pH, but rather with the absence of buffering salts in the water. This hypothesis does not, however, explain why standard buffer solution at pH 4.0 resulted in a "non-specific" positive test result. It is clear from the foregoing preliminary data that further studies are required to determine whether problems associated with the use of unbuffered samples in ICA systems are batch-specific and originate in the manufacturing process, or, whether they are inherent in the ICA technology.

ECLA was one of the identification technologies used to identify aerosol disseminated simulants at JFT II at DPG in September 1995 [2]. Comparison of the results from JFT II and IV revealed that ECLA performance at JFT IV was apparently

superior to that at JFT II. At JFT II, the agent disseminated was correctly identified by ECLA in 26/38 trials (68%) compared to 100% correct identification of disseminated agents at JFT IV. However, there were some important differences in trial protocol between JFT II and IV which may have influenced the outcome of ECLA analyses. At JFT II, four simulants were used in the trials compared to the use of two simulants at JFT IV, hence there would have been a higher probability of obtaining a correct identification at JFT IV than at JFT II. At JFT IV, buffer was used to determine the cut-off thresholds for ECLA and this contributed to a higher number of positive trial identifications compared to JFT II where cut-off thresholds were based on ECLA of pre-alarm samples. However, probably the most important factor contributing to a higher rate of correct identifications at JFT IV compared to JFT II was the use at JFT IV of redundant collectors. At JFT IV, several CIBADS collectors were deployed at different positions both on and off the test grid, compared to JFT II where identification was reliant upon samples collected by a single collector located at one fixed grid position.

CONCLUSIONS

Two Sentry systems, CIBADS V96:XXM and CIBADS V97:LVAS, and the CDU:LVAS were deployed at different locations at JFT IV for the detection and collection of aerosolized simulants, BG and EH. Samples collected by these Sentries were analysed for the presence of BG and EH, both by ECLA and by ICA. By ECLA, BG was identified in 100% of trials when BG was the disseminated agent. Similarly, in 100% of the trials in which EH was sprayed, EH was identified in one or more samples. The amount of BG and EH in test samples was estimated from standard reference curves and ranged from 7×10^4 to 7×10^6 cfu/ml for BG and 1×10^3 to 1×10^4 cfu/ml for EH. In 40% of trials, trace amounts of the other JFT IV simulant were detected in addition to the agent disseminated; factors which may have contributed to this phenomenon are discussed. For a given collection period, there was a high correlation between the amount of simulant identified by ECLA in test samples and that collected by NBSS at reference towers, thus suggesting that the cloud presented to the referee samplers was a good representation of the cloud

presented to the CIBADS samplers. By ICA, the BG tickets gave "false positive" test results with the water used as collection fluid for the CIBADS samplers and, although the BG tickets were shown to be sensitive enough to detect BG in the concentrations estimated to be present in test samples, these tickets were declared unreliable. The EH tickets did not react non-specifically with water but were shown to be insufficiently sensitive to detect EH in the concentrations estimated to be present in test samples.

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Table I
JFT IV trial schedule and window times

Trial No.	Date	Session No.	Trial Window	
			Open	Closed
1	971022	I	20:52:26	Not known
2	971022		22:54:00	23:28:16
3	971023	II	20:28:00	20:51:32
4	971026	III	19:36:20	20:13:00
5	971026		20:52:00	21:29:00
6	971026		22:00:00	22:51:31
7	971026		23:18:00	23:51:00
8	971027		00:13:00	00:48:00
9	971027		01:22:00	01:45:00
10	971027	IV	16:37:00	17:20:00
11	971028	V	21:22:00	22:28:00
12	971029	VI	19:16:00	19:54:13
13	971030		01:10:00	01:37:00
14	971030	VII	20:09:30	20:46:01
15	971030		21:18:00	22:05:00
16	971030		22:31:00	23:09:17
17	971030		23:55:00	00:39:48
18	971031		01:03:00	01:43:00
19	971031		02:06:00	02:40:00
20	971031		03:05:00	03:33:00
21	971031		03:55:00	04:30:00
22	971031	VIII	21:45:00	22:14:00
23	971031		22:43:00	23:25:00
24	971101		00:02:00	00:47:00
25	971106	IX	19:40:00	20:05:00

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Table II
Electrochemiluminescence immunoassay analysis of trial samples: *Bacillus subtilis*

Trial No.	V96: XMX		V97: LVAS		CDU: LVAS		
	S/N ^a	Result ^b	S/N	Result	Sample No.	S/N	Result
1	No sample		No sample		15	22.6	+
2	1.5	+	1.8	+	No sample		
3	3.2	+	5.5	+	24, 26	2.9, 9.4	+, +
4	4.0	+	8.2	+	32, 35	5.5, 9.7	+, +
5	No sample		2.0	+	No sample		
6	5.6 ^a	+	4.6	+	No sample		
7	2.8	+	No sample		49, 52, 53	2.8*, 25.5, 29.2	+, +, +
8	7.5	+	9.3	+	56, 58, 59	3.0, 12.3, 9.5	+, +, +
9	2.8	+	No sample		No sample		
10	No sample		8.0	+	64, 66, 67	1.0, 5.9, 6.4	-, +, +
11	11.4	+	No sample		70, 72	9.2, 26.0	+, +
12	2.7	+	9.2	+	75, 77	3.3, 5.3	+, +
13	5.2	+	9.6	+	79, 81	8.0, 18.1	+, +
14	10.4	+	14.0	+	84, 86	4.4, 17.1	+, +
15	6.0	+	11.5	+	90, 92	1.6, 15.8	+, +
16	7.2	+	18.2	+	96, 98	2.5, 10.0	+, +
17	No sample		3.4	+	105, 107	2.3, 2.3	+, +
18	2.8	+	8.8	+	111, 113	1.2, 14.0	+, +
19	1.6	+	5.0	+	114, 116	10.0, 14.7	+, +
20	3.3	+	9.0	+	119, 121	2.6, 8.3	+, +
21	No sample		2.7	+	2, 4	3.9, 2.7	+, +
22	No sample		17.6	+	6, 8	10.7, 7.5	+, +
23	No sample		16.2	+	12, 15	2.8, 12.1	+, +
24	No sample		12.9	+	18, 22	10.8, 15.2	+, +
25	10.4	+	9.6	+	25, 27	6.1, 11.6	+, +

* Mean and standard deviation of S calculated from two tubes of test sample

^a S/N: (Mean ECL of triplicate tubes of test sample) divided by (mean ECL of triplicate tubes of negative control sample plus two standard deviations)

^b Positive test (+) indicated by S/N > 1.0; negative test (-) indicated by S/N = or < 1.0

Table III
Electrochemiluminescence immunoassay analysis of trial samples: *Erwinia herbicola*

Trial No.	V96: XMX		V97: LVAS		CDU: LVAS		
	S/N ¹⁰	Result ¹¹	S/N	Result	Sample No.	S/N	Result
1	No sample		No sample		15	0.9	-
2	3.0	+	13.7	+	No sample		
3	0.9	-	1.0	-	24, 26	0.9, 1.0	-,-
4	1.0	-	1.0	-	32, 35	[1.1], 1.0	-,-
5	No sample		1.3	+	No sample		
6	[1.2]	-	1.0	-	No sample		
7	0.9	-	No sample		49, 52, 53	1.0, [1.4], 0.9	-,-,-
8	0.9	-	[1.1]	-	56, 58, 59	[1.2], [1.1], [1.1]	-,-,-
9	[2.0]	-	No sample		No sample		
10	No sample		1.0	-	64, 66, 67	[1.2], 1.3, [1.1]	-,+,-
11	[1.4]	-	No sample		70, 72	1.0, [1.2]	-,-
12	[1.2]	-	1.1	+	75, 77	[1.1], [1.6]	-,-
13	[1.1]	-	1.0	-	79, 81	1.1, [1.3]	+,-
14	1.0	-	[1.1]	-	84, 86	0.9, [1.1]	-,-
15	0.9	-	0.9	-	90, 92	0.8, 1.0	-,-
16	[1.3]	-	[1.1]	-	96, 98	[1.4], 0.9	-,-
17	No sample		1.4	+	105, 107	1.0, 3.5	-,+
18	[1.3]	-	1.4	+	111, 113	[1.4], [1.5]	-,-
19	1.4	+	[1.5]	-	114, 116	1.5, 1.3	+,+
20	1.4	+	1.7	+	119, 121	[1.9], 1.4	-,+
21	No sample		1.0	-	2, 4	[1.2], 1.3	-,+
22	No sample		[1.1]	-	6, 8	0.9, [1.1]	-,-
23	No sample		0.9	-	12, 15	0.9, 0.9	-,-
24	No sample		1.0	-	18, 22	[1.4], 1.0	-,-
25	0.8	-	0.6	-	25, 27	0.9, 0.7	-,-

[] Brackets indicate S was not significantly different from N i.e., the mean ECL minus two standard deviations was less than or equal to N plus two standard deviations.

¹⁰ S/N: As in Table II

¹¹ Result: As in Table II

Table IV
Estimated concentrations of *Bacillus subtilis* in trial samples

Trial No.	V96:XXM		V97:LVAS		CDU:LVAS		
	ug/ml	cfu/ml ¹²	ug/ml	cfu/ml	Sample no.	ug/ml	cfu/ml
1	No sample		No sample		15	9.198	9.2x10E6
2	0.078	7.8x10E4	0.084	8.4x10E4	No sample		
3	0.096	9.6x10E4	0.180	1.8x10E5	24, 26	0.089, 0.516	8.9x10E4, 5.2x10E5
4	0.121	1.2x10E5	0.369	3.7x10E5	32, 35	0.183, 0.552	1.8x10E5, 5.5x10E5
5	No sample		0.087	8.7x10E4	No sample		
6	0.145	1.5x10E5	0.117	1.2x10E5	No sample		
7	0.092	9.2x10E4	No sample		49, 52, 53	0.092, 3.614, 6.547	9.2x10E4, 3.6x10E6, 6.6x10E6
8	1.050	1.1x10E6	2.070	2.1x10E6	56, 58, 59	0.177, 6.770, 2.238	1.8x10E6, 6.8x10E6, 2.2x10E6
9	0.159	1.6x10E5	No sample		No sample		
10	No sample		1.250	1.3x10E6	64, 66, 67	neg, 0.553, 0.665	neg, 5.5x10E5, 6.7x10E5
11	0.370	3.7x10E5	No sample		70, 72	0.258, 3.865	2.6x10E5, 3.9x10E6
12	0.086	8.6x10E4	0.492	4.9x10E5	75, 77	0.101, 0.170	1.0x10E5, 1.7x10E5
13	0.334	3.3x10E5	0.834	8.3x10E5	79, 81	0.602, 4.743	6.0x10E5, 4.7x10E6
14	0.971	9.7x10E5	2.058	2.1x10E6	*4, 86	0.289, 3.879	2.9x10E5, 3.9x10E6
15	0.398	4.0x10E5	1.235	1.2x10E6	90, 92	0.162, 2.991	1.6x10E5, 3.0x10E6
16	0.242	2.4x10E5	2.270	2.3x10E6	96, 98	0.092, 0.429	9.2x10E4, 4.3x10E5
17	No sample		0.119	1.2x10E5	105, 107	0.094, 0.095	9.4x10E4, 9.5x10E4
18	0.098	9.8x10E4	0.338	3.4x10E5	111, 113	0.072, 0.972	7.2x10E4, 9.7x10E5
19	0.078	7.8x10E4	0.154	1.5x10E5	114, 116	0.425, 1.104	4.3x10E5, 1.1x10E6
20	0.088	8.8x10E4	0.296	3.0x10E5	119, 121	0.077, 0.255	7.7x10E4, 2.6x10E5
21	No sample		0.104	1.0x10E5	2, 4	0.136, 0.104	1.4x10E5, 1.0x10E5
22	No sample		1.010	1.0x10E6	6, 8	0.328, 0.197	3.3x10E5, 2.0x10E5
23	No sample		0.817	8.2x10E5	12, 15	0.088, 0.413	8.8x10E4, 4.1x10E5
24	No sample		0.467	4.7x10E5	18, 22	0.331, 0.687	3.3x10E5, 6.9x10E5
25	0.397	4.0x10E5	0.337	3.4x10E5	25, 27	0.161, 0.512	1.6x10E5, 5.1x10E5

¹² 1 pg = approximately 1 cfu/ml

Table V
Estimated concentrations of *Erwinia herbicola* in trial samples

Trial No.	V96: XMX cfu/ml	V97: LVAS cfu/ml	CDU: LVAS	
			Sample No.	cfu/ml
2	1.7x10E3	0.8x10E4	No sample	
5	No sample	1.3x10E3	No sample	
10	No sample	¹³	64, 66, 67	-, 5.4x10E2, -
12	-	4.5x10E2	75, 77	-, -
13	-	-	79, 81	4.6x10E3, -
17	No sample	1.4x10E3	105, 107	-, 1.9x10E3
18	No sample	5.6x10E2	111, 113	-, -
19	5.5x10E2	-	114, 116	6.1x10E2, 5.3x10E2
20	5.6x10E2	7.2x10E2	119, 121	-, 5.6x10E2
21	No sample	-	2, 4	-, 1.4x10E3

¹³ -: Negative

Table VI
 Immunochemographic analysis¹⁴ of trial samples: *Bacillus subtilis* and *Erwinia herbicola*

Trial No.	V96: XMX		V97: LVAS		CDU: LVAS		
	BG	EH	BG	EH	Sample No.	BG	EH
1	No sample		No sample		15	+	-
2	+	-	+	-	No sample		
3	+	-	+(w)	-	24, 26	+(w), +(w)	-,-
4	+(w)	-	+	-	32, 35	+, +	-,-
5	No sample		+	-	No sample		
6	+(w)	-	+	-	No sample		
7	+	-	No sample		49, 52, 53	+, +, +	-,-,-
8	+	-	+	-	56, 58, 59	+, +, +	-,-,-
9	+	-	No sample		No sample		
10	No sample		+	-	64, 66, 67	+, +, +	-,-,-
11	+	-	No sample		70, 72	+, +	-,-
12	+	-	+	-	75, 77	+, +	-,-
13	+	-	+	-	79, 81	+, +	-,-
14	+	-	+	-	84, 86	+, +	-,-
15	+	-	+	-	90, 92	+(w), +	-,-
16	+	-	+	-	96, 98	+, +	-,-
17	No sample		+	-	105, 107	+(w), +(w)	-,-
18	+(w)	-	+(w)	-	111, 113	+, +	-,-
19	-	-	+	-	114, 116	+, +	-,-
20	+	-	+	-	119, 121	+, +	-,-
21	No sample		+	-	2, 4	+, +(w)	-,-
22	No sample		+	-	6, 8	+, +	-,-
23	No sample		+	-	12, 15	+, +	-,-
24	No sample		+	-	18, 22	+, +	-,-
25	+	-	+	-	25, 27	+, +	-,-

¹⁴ ICA on V97: LVAS samples performed on trial site at DPG; ICA on V96: XMX and CDU: LVAS samples performed at DRES following JFT IV

Table VII
Electrochemiluminescence immunoassay and immunochromatographic analysis
of water samples

Sample	ECLA				pH	BG ICA	
	BG		EH			Before buffering	After buffering ¹
	S/N	Result	S/N	Result			
CIBADS NANOpure water ¹⁶ (before filter change)	1.6	+ (trace)	0.7	-	ND	+	-
CIBADS NANOpure water (after filter change)	1.6	+ (trace)	0.9	-	7.4	+	-
DRES Bldg 1, R415 water ¹⁷	0.8	-	0.6	-	7.0	+	-
DRES Bldg 1, R402 water ¹⁸	[1.1]	-	0.6	-	7.4	+	-
DRES Bldg 1, R 102 3X distilled water ¹⁹	[1.1]	-	0.6	-	7.5	+	-
DRES Bldg 1, R 203, NANOpure water	[1.3]	-	0.6	-	7.3	+	-
DRES Bldg 1, R 209 1X distilled tap water	[1.2]	-	0.7	-	7.0	+	-
DRES Bldg 1, R 209 hard tap water	1.6	+ (trace)	[1.0]	-	8.0	+	-
DRES Bldg 67, MilliQ water ²⁰	[1.1]	-	0.6	-	7.0	+	-
DRES Bldg 67, NANOpure water ²¹	1.1	+ (trace)	0.7	-	7.3	+	-
Buffer pH standard ²²	Not done				4.0	+	Not done
Buffer pH standard ²³	Not done				7.0	-	Not done
Buffer pH standard ²⁴	Not done				10.0	-	Not done

¹⁵ Samples were buffered by the addition of tablet PBS, Dulbecco A to 100 ml water sample

¹⁶ NANOpure II water deionization system with hollow fibre filter, Sybron, Barnstead/Thermolyne Corp., Dubuque, IO

¹⁷ Water deionization system, Sybron, Barnstead Inc.

¹⁸ Corning Mega Pure MP-6A water still and deionization system, Corning Glass Works, Corning, NY

¹⁹ Corning AG11 water still, Corning Glass Works (Corning Glass Works)

²⁰ MilliQ UF Plus ultrapure water filtration system, Millipore Inc., Bedford, MA

²¹ See footnote 20.

²² Potassium biphthalate buffer, 0.05M, Fisher Scientific, Nepean, ON

²³ Potassium monobasic-sodium hydroxide buffer, 0.05M (Fisher Scientific)

²⁴ Potassium carbonate, potassium borate, potassium hydroxide buffer, 0.05M (Fisher Scientific)

Table VIII
Comparison of simulant concentrations collected by referee New Brunswick silt sampler
and CIBADS V96:XXM²⁵ at reference tower 2

Trial No.	Agent Sprayed	FLAPS Trigger On - Off hr: min: sec	XXM Sampler On -Off ²⁶ hr: min: sec	Concentration of Simulant		
				V96:XXM cfu/ml	Referee NESS ACPLA ²⁷	
					Peak	Total
3	BG	20:35:00 - 20:36:17 20:37:22 - 20:45:17	FLAPS ²⁸	9.6x10E4	23.3	870.8
4	BG	19:54:36 - 19:56:30	19:54:00 - 20:13:07	1.2x10E5	90.6 *	1665.9*
6	BG	22:34:04 - 22:37:09	22:34:00 - 22:39:38	1.5x10E5	14.8*	244.3*
7	BG	23:32:40 - 23:33:50 23:38:53 - 23:40:49	23:32:00 - 23:47:00	9.2x10E4	11.1	194.5.0
8	BG	00:23:09 - 00:27:34 00:28:34 - 00:38:10	00:24:06-00:39:00	1.1x10E6	125.6*	4621.4*
9	BG	01:28:51 - 01:33:23	01:29:00 - 01:36:00	1.6x10E5	57.2	1635.2
11	BG	21:36:57 - 21:39:13	21:38:00 - 21:41:53	3.7x10E5	54.1	614.8
12	BG	19:31:09 - 19:36:53	19:31:50 - 19:38:05	8.6x10E4	66.8	1793.1
13	BG	01:19:31 - 01:24:41	01:19:55 - 01:27:29	3.3x10E5	95.4	1879.5
14	BG	20:22:22 - 20:29:26	20:22:42 - 20:31:32	9.7x10E5	124.0	5652.3
15	BG	21:37:43 - 21:48:35	21:38:08 - 21:49:00	4.0x10E5	13.8	3292.0
16	BG	22:46:31 - 22:57:55	22:46:55 - 22:58:07	2.4x10E5	83.2	5231.4
18	BG	01:19:53 - 01:23:53	01:20:25 - 01:25:30	9.8x10E4	38.7*	1202.1*
19	BG	02:14:52 - 02:17:16 02:20:59 - 02:23:39	02:16:30 - 02:20:02	7.8x10E4	8.0	50.9
20	BG	03:14:53 - 03:19:38	03:15:09 - 03:21:16	8.8x10E4	77.4	2424.9
25	BG	19:50:22 - 19:56:16	19:50:38 - 19:56:16	4.0x10E5	80.6	3203.5

* Data used to determine peak and total ACPLA were derived from time intervals less than those during which the XXM sampler was operational, as follows: Trial 4: 19:54:00 - 20:08:45; trial 6: 22:34:29 - 22:39:38; trial 8: 00:24:06 - 00:37:30; trial 18: 01:20:49 - 01:25:30.

²⁵ Data included only for trials in which V96:XXM samples were taken

²⁶ XXM sampler on - off times provided by M. Spence (personal communication)

²⁷ ACPLA taken from referee data were provided by DPG. Unless otherwise noted (*), peak and total ACPLA were calculated from data available for the full interval of sampling.

²⁸ Sampler on - off times assumed to be same as FLAPS trigger on - off times

Table IX
Comparison of simulant concentrations collected by referee New Brunswick slit sampler
and CIBADS V97:LVAS²⁹ at reference tower 3

Trial No.	Agent Sprayed	FLAPS Trigger On - Off hr: min: sec	LVAS Sampler On - Off ³⁰ hr: min: sec	Concentration of Simulant ³¹		
				V97:LVAS (cfu/ml)	Referee NBSS ACPLA ³²	
					Peak	Total
3	BG	20:35:03 - 20:36:20 20:37:39 - 20:43:15	20:35:03 - 20:45:15	1.8x10E5	20.1	771.2
4	BG	19:54:43 - 19:58:36	19:54:43 - 20:04:43	3.7x10E5	94.3	1917.1
5	EH	21:03:30 - 21:06:37 21:11:45 - 21:19:17	21:03:30 - 21:13:30	1.3x10E3	2.7	67.8
6	BG	22:34:06 - 22:38:28	22:34:06 - 22:44:06	1.2x10E5	31.8*	555.5*
8	BG	00:23:19 - 00:26:13 00:28:54 - 00:37:38	00:23:19 - 00:33:19	2.1x10E6	114.0	5719.6
10	BG	17:01:09 - 17:10:31	17:01:09 - 17:11:09	1.3x10E6	57.8	2043.3
12	BG	19:31:53 - 19:37:41	19:31:53 - 19:41:53	4.9x10E5	64.7	2412.7
13	BG	01:20:04 - 01:26:04	01:20:04 - 01:30:04	8.3x10E5	90.6	1967.0
14	BG	20:22:22 - 20:30:05	20:22:22 - 20:32:22	2.1x10E6	127.7	7826.5
15	BG	21:37:45 - 21:49:17	21:37:45 - 21:47:45	1.2x10E6	59.9	3931.3
16	BG	22:46:45 - 22:58:02	22:46:45 - 22:56:45	2.3x10E6	77.9*	5189.0*
17	EH	00:24:44 - 00:28:52	00:24:44 - 00:34:44	1.4x10E3	6.9	272.4
18	BG	01:20:14 - 01:24:16	01:20:14 - 01:30:14	3.4x10E5	71.0*	1924.6*
19	BG	02:15:39 - 02:17:35 02:21:12 - 02:24:27	02:15:39 - 02:25:39	1.5x10E5	25.4	535.3
20	BG	03:14:07 - 03:19:55	03:14:07 - 03:24:07	2.0x10E5	63.6*	1967.5*
22	BG	21:53:47 - 22:01:07	21:53:47 - 22:03:47	1.0x10E6	88.5	4480.4
23	BG	22:55:07 - 23:10:49	22:55:07 - 23:05:07	8.2x10E5	104.9*	5263.3*
25	BG	19:50:00 - 19:56:06	19:50:00 - 20:05:00	3.4x10E5	89.0*	3558.7*

* Data used to determine peak and total ACPLA were derived from time intervals less than those during which the LVAS sampler was operational, as follows: trial 6: 22:34:29 - 22:44:06; trial 16: 22:46:52 - 22:56:45; trial 18: 01:20:49 - 01:30:14; trial 20: 03:14:21 - 03:24:07; trial 23: 22:57:04 - 23:05:07; trial 25: 19:50:00 - 20:02:48.

²⁹ Only trials in which V97:LVAS samples were taken and ACPLA data were available are included. In addition, trial 21 was omitted because the V97:LVAS sample was negative by ECLA for EH.

³⁰ Assume sampling start time same as FLAPS Trigger On time and period of sampling 10 minutes from FLAPS Trigger On time (C. Boulet, personal communication).

³¹ Identity of simulant in NBSS and V97:LVAS samples was assumed to be same as simulant sprayed. Concentrations of simulants in V97:LVAS samples were derived from ECLA analyses. Concentrations of simulants in NBSS samples were taken from ACPLA data provided by DPG.

³² ACPLA were taken from referee data provided by DPG. Unless otherwise noted (*), peak and total ACPLA were determined from data available for the full interval of sampling.

Table X
Simulants sprayed in relation to simulants identified by electrochemiluminescence in
V96:XXM, V97:LVAS, and CDU:LVAS

Tris No.	Agent Sprayed	V96:XXM	V97:LVAS	Sample No.	CDU:LVAS	Ident'n of Simulant Sprayed ³³	Ident'n of Other JFT IV Simulant ³⁴
1	BG	No sample	No sample	15	BG	Yes	No
2	EH	BG, EH	BG, EH	No sample		Yes	Yes
3	BG	BG	BG	24, 26	BG, BG	Yes	No
4	BG	BG	BG	32, 35	BG, BG	Yes	No
5	EH	No sample	BG, EH	No sample		Yes	Yes
6	BG	BG	BG	No sample		Yes	No
7	BG	BG	No sample	49, 52, 53	BG, BG, BG	Yes	No
8	BG	BG	BG	56, 58, 59	BG, BG, BG	Yes	No
9	BG	BG	No sample	No sample		Yes	No
10	BG	No sample	BG	64, 66, 67	-, BG/EH, BG	Yes	Yes
11	BG	BG	No sample	70, 72	BG, BG	Yes	No
12	BG	BG	BG, EH	75, 77	BG, BG	Yes	Yes
13	BG	BG	BG	79, 81	BG/EH, BG	Yes	Yes
14	BG	BG	BG	84, 86	BG, BG	Yes	No
15	BG	BG	BG	90, 92	BG, BG	Yes	No
16	BG	BG	BG	96, 98	BG, BG	Yes	No
17	EH	No sample	BG, EH	105, 107	BG, BG/EH	Yes	Yes
18	BG	BG	BG, EH	111, 113	BG, BG	Yes	Yes
19	BG	BG, EH	BG	114, 116	BG/EH, BG/EH	Yes	Yes
20	BG	BG, EH	BG, EH	119, 121	BG, BG/EH	Yes	Yes
21	EH	No sample	BG	2, 4	BG, BG/EH	Yes	Yes
22	BG	No sample	BG	6, 8	BG, BG	Yes	No
23	BG	No sample	BG	12, 15	BG, BG	Yes	No
24	BG	No sample	BG	18, 22	BG, BG	Yes	No
25	BG	BG	BG	25, 27	BG, BG	Yes	No

³³ Identification of agent sprayed at one or more grid sites by one or more identification methods

³⁴ Identification, at one or more grid sites by one or more identification methods, of agent in addition to agent sprayed

Figures 1 - 6. Estimation of concentration of *Bacillus subtilis* in JFT IV trial samples by ECLA. Samples collected by CIBADS V96:XXMX, V97:LVAS and the CDU:LVAS were assayed in parallel by trial number (T). Positive control samples (+_+) consisted of *Bacillus subtilis* at 10.0, 1.0 and 0.1 ug/ml protein; negative control samples (....) consisted of all assay reagents except antigen. Data points are the mean of triplicate determinations. Error bars represent two standard deviations above and below the mean. *Bacillus subtilis* concentrations were estimated from log regression curves derived from mean ECL counts in positive control tubes. CDU number refers to CDU LVAS sample number.

- Figure 1: a: T2 and T5; b: T3; c: T4; and d: T6;
Figure 2: a: T7; b: T8; c: T9 and T10; and d: T11;
Figure 3: a: T12; b: T13; c: T14; and d: T15;
Figure 4: a: T16; b: T17; c: T18; and d: T19;
Figure 5: a: T20; b: T21; c: T22; and d: T23;
Figure 6: a: T24 and b: T25

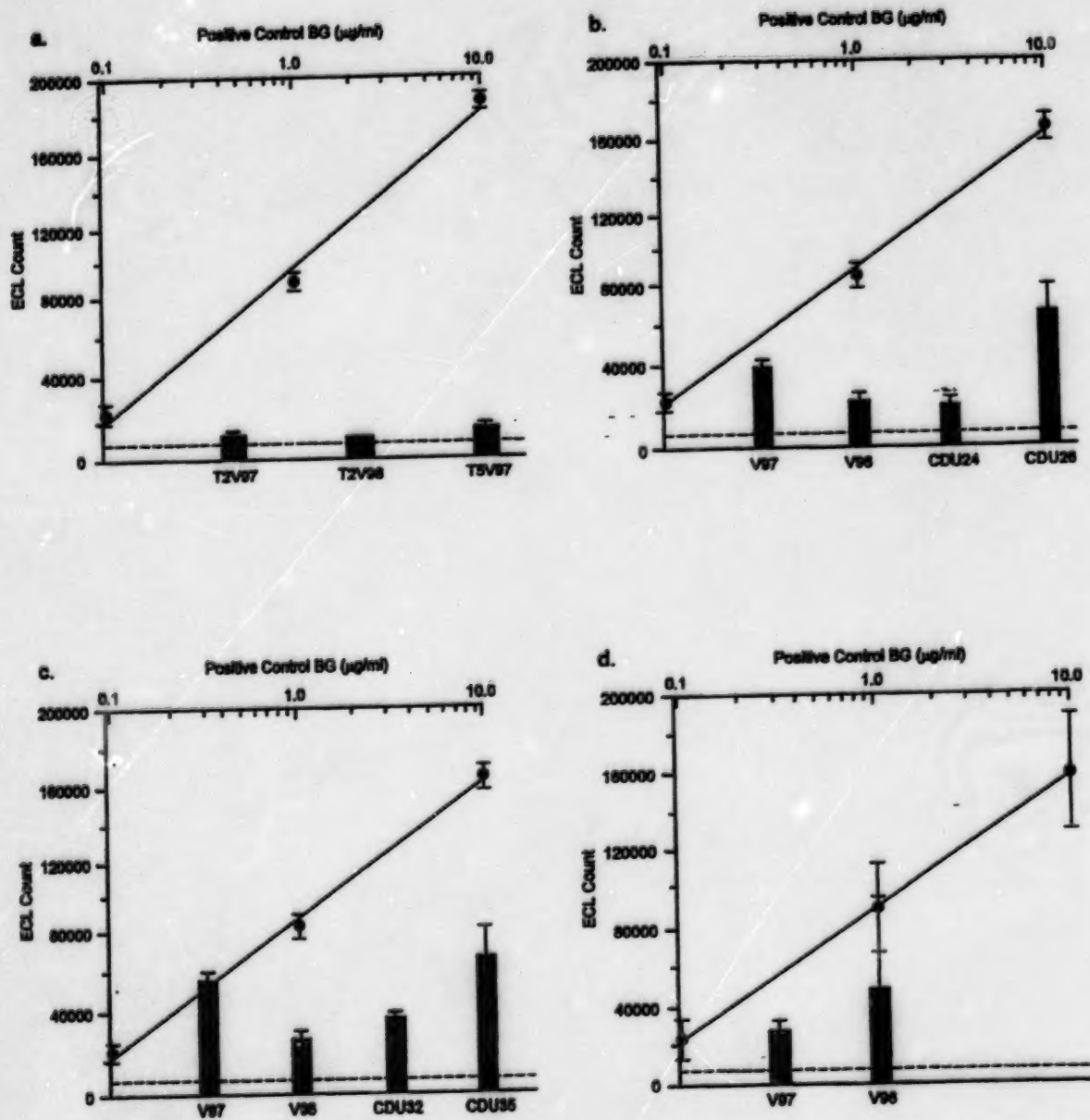


Figure 1 Estimation of concentration of *Bacillus subtilis* in JFT IV trial samples by ECLA.
a.: T2 and T5; b.: T3; c.: T4; and d.: T6

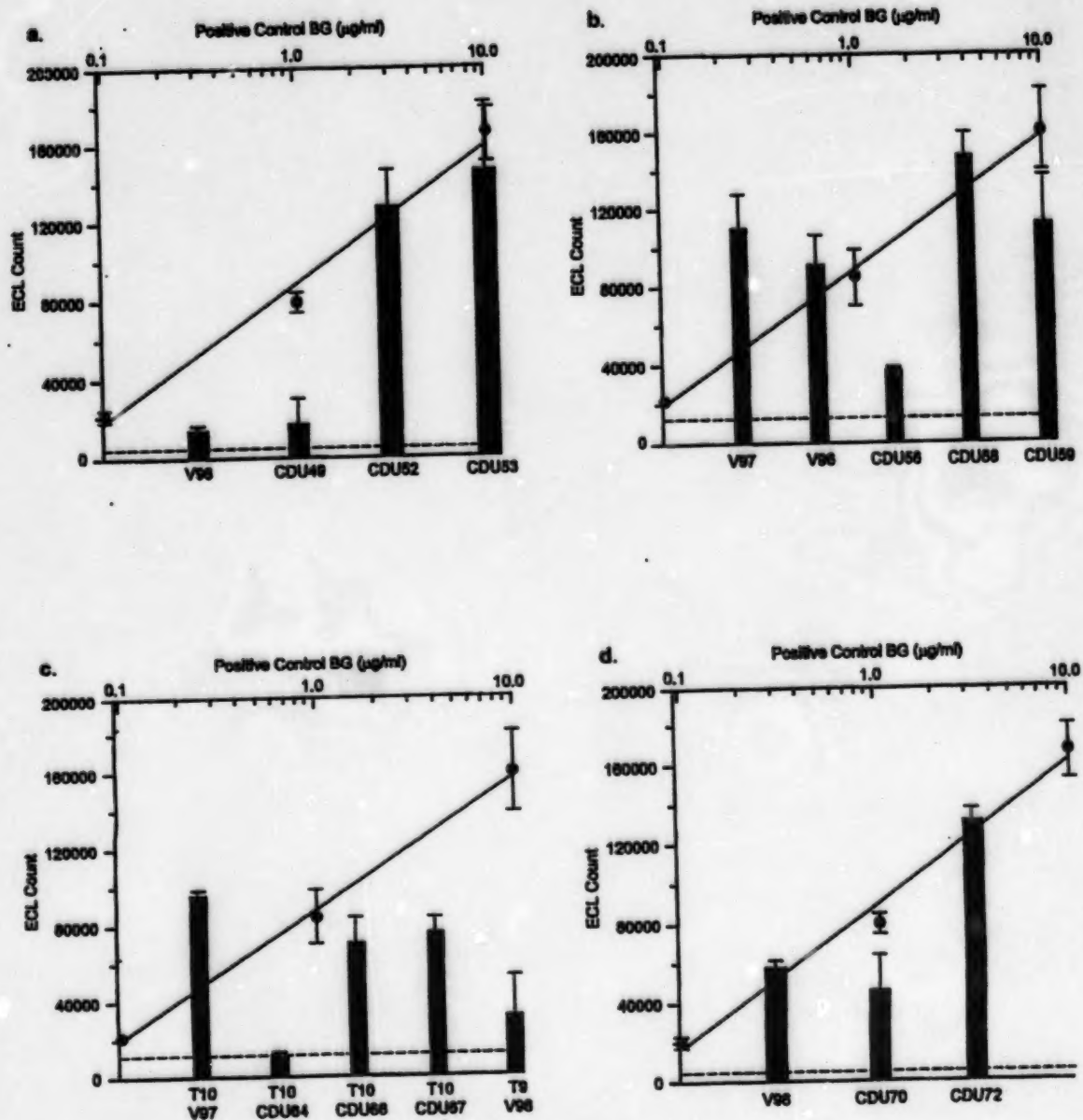


Figure 2

Estimation of concentration of *Bacillus subtilis* in JFT IV trial samples by ECLA.
 a.: T7; b.: T8; c.: T9 and T10; and d.: T11

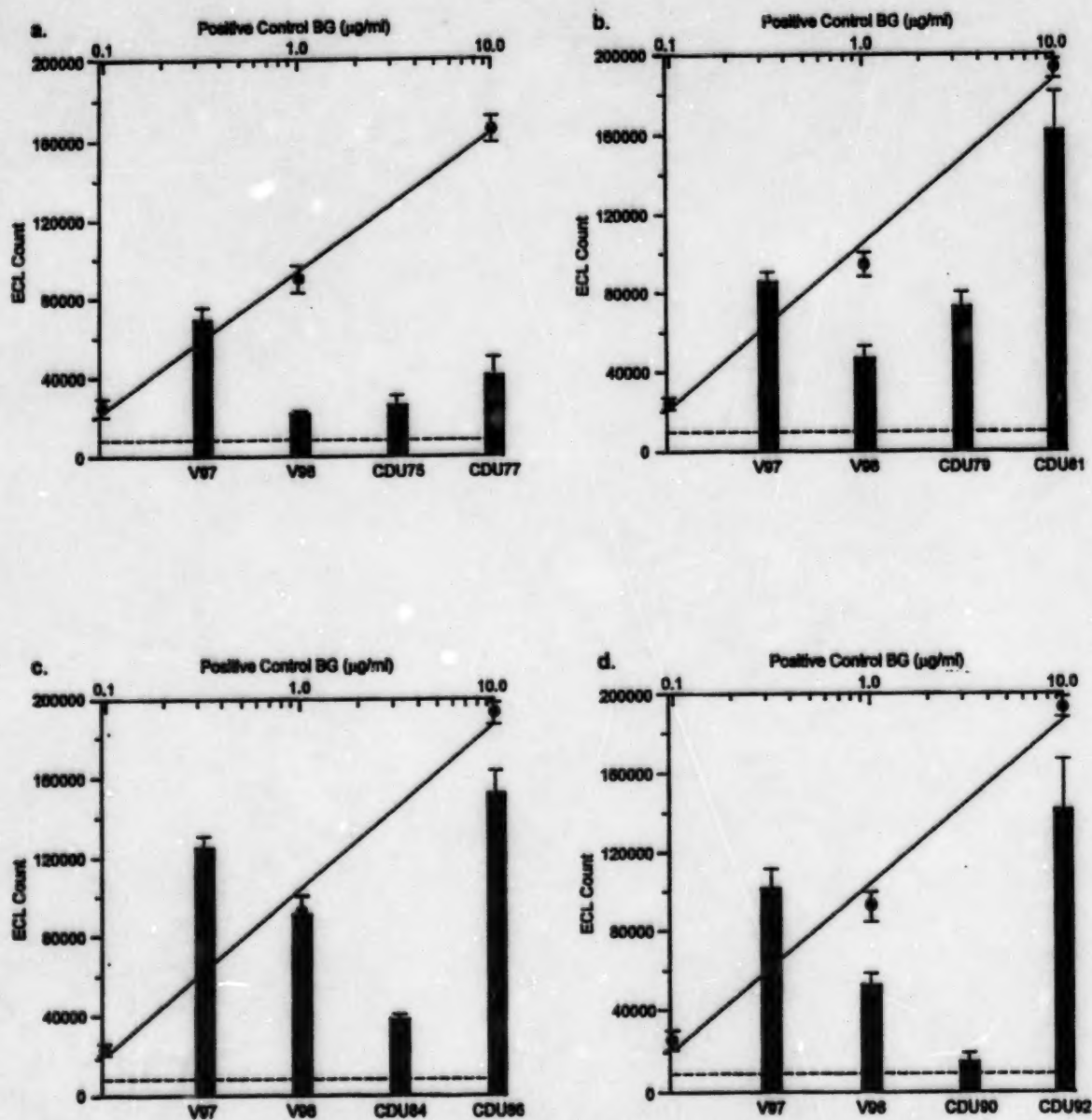


Figure 3

Estimation of concentration of *Bacillus subtilis* in JFT IV trial samples by ECLA.
a.: T12; b.: T13; c.: T14; and d.: T15

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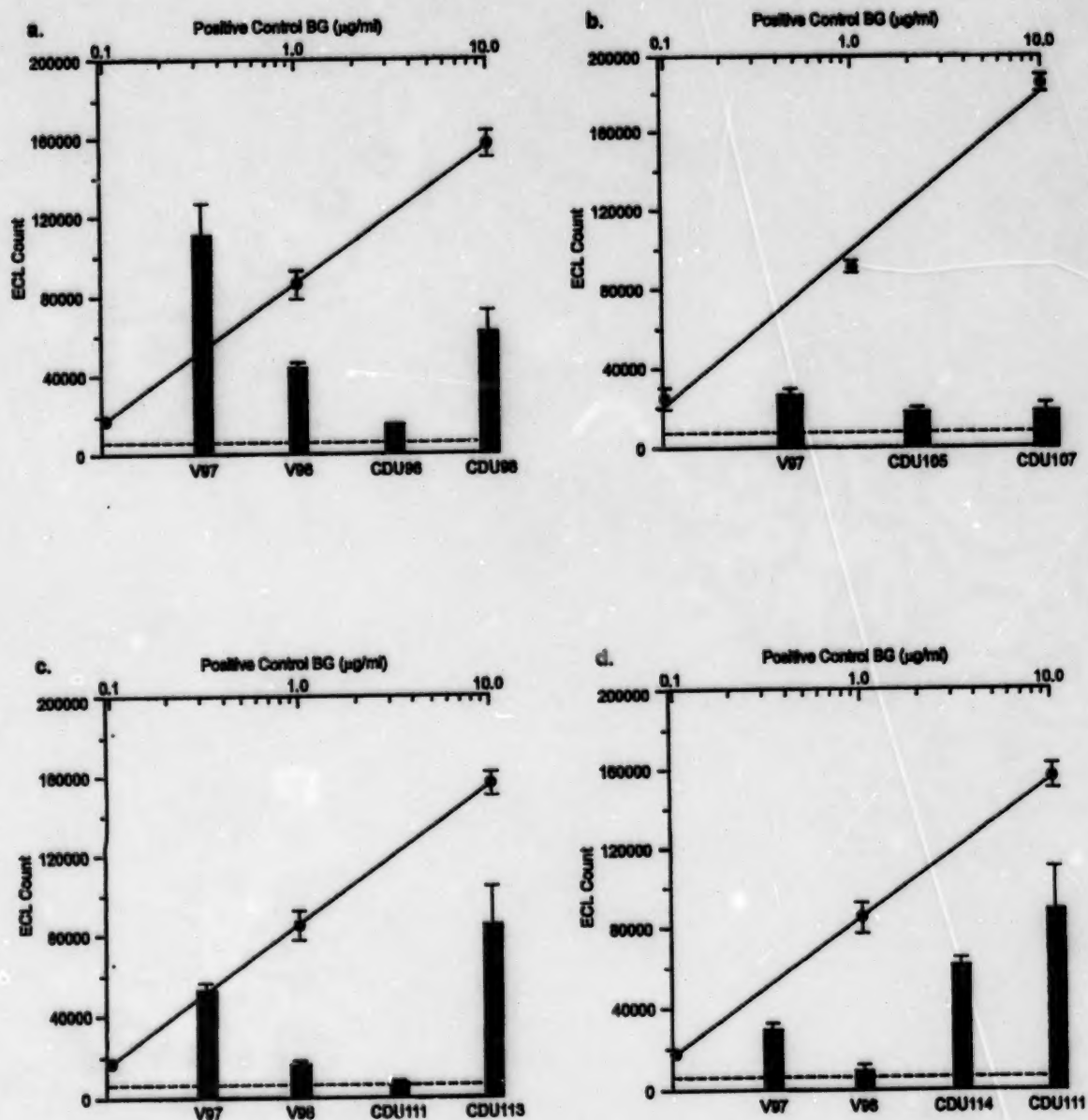


Figure 4

Estimation of concentration of *Bacillus subtilis* in JFT IV trial samples by ECLA.
a.: T16; .b: T17; c.: T18; and d.: T19

UNCLASSIFIED

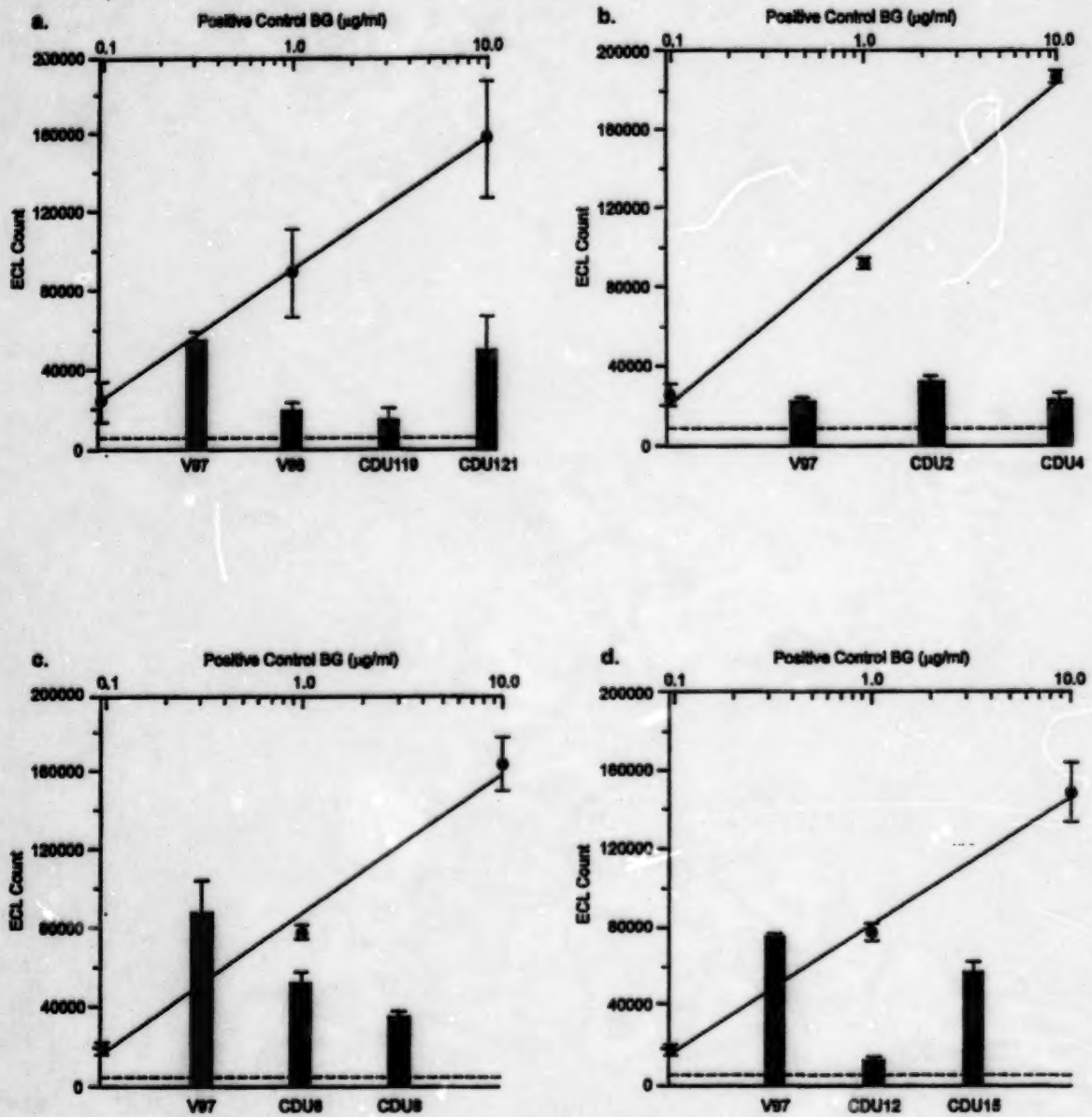


Figure 5 Estimation of concentration of *Bacillus subtilis* in JFT IV trial samples by ECLA.
a.: T20; b.: T21; c.: T22; and d.: T23

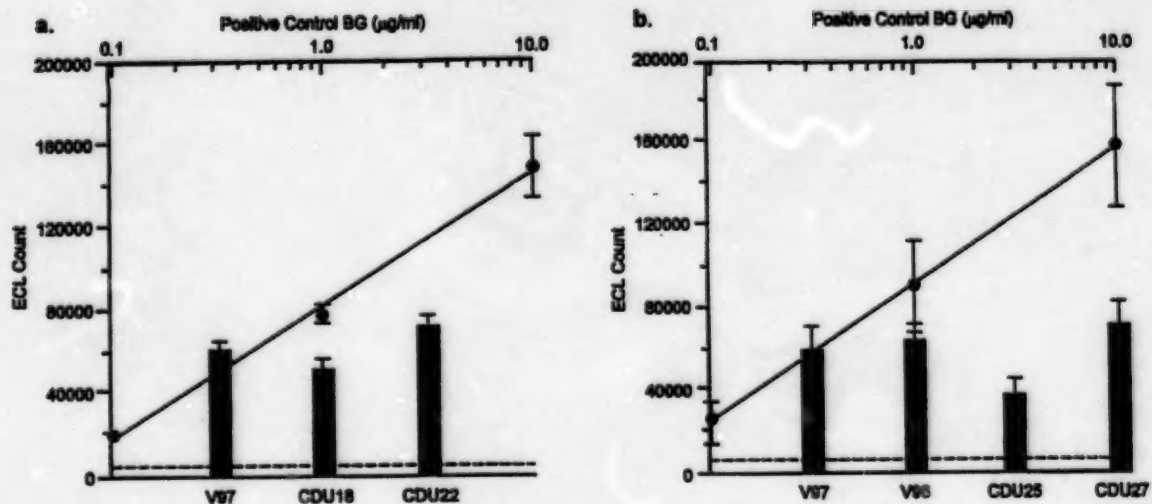
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Figure 6 Estimation of concentration of *Bacillus subtilis* in JFT IV trial samples by ECLA.
a.: T24; and b.: T25

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This report documents the analysis of samples taken at Joint Field Trials (JFT) IV for two biological agent simulants, *Bacillus subtilis* var. *niger* ssp. *globigii* (BG) and *Erwinia herbicola* (EH), by two different identification technologies, electro-chemiluminescence immunoassay (ECLA) and immunochromatographic assay (ICA). In each of 25 trials, BG or EH was disseminated and collected by one or more CIBADS collector systems, which consisted of two prototype CIBADS Sentry units located at different positions on the test grid and a Concept Demonstrator unit located outside the grid. On analysis of samples by ECLA, BG and EH were identified in 100% of trials in which BG and EH, respectively, were disseminated. The amount of simulant in samples was estimated from standard concentration curves. BG was found to be present in samples in concentrations ranging from approximately 7×10^4 to 7×10^6 cfu/mL; the concentration of EH in samples ranged from approximately 1×10^3 to 1×10^4 cfu/mL. For a given collection period, the amount of simulant identified by ECLA in samples was positively correlated with the amount of simulant collected by referee samplers. On analysis of samples by ILA, BG tickets produced "false positive" reaction lines when tested with CIBADS collection fluid (water) and, although shown to be sensitive enough to detect BG in the concentrations estimated to be present in samples, these tickets were declared unreliable. EH tickets did not react non-specifically when tested with water but were shown to be insufficiently sensitive to detect EH in the concentrations estimated to be present in samples. The implications of the findings documented in this report of are discussed.

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Joint Field Trials

electrochemiluminescence immunoassay (ECLA)

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biological agent simulants

Bacillus subtilis var. *niger* ssp. *globigii* (BG)

Erwinia herbicola (EH)